

FIGURE 1A

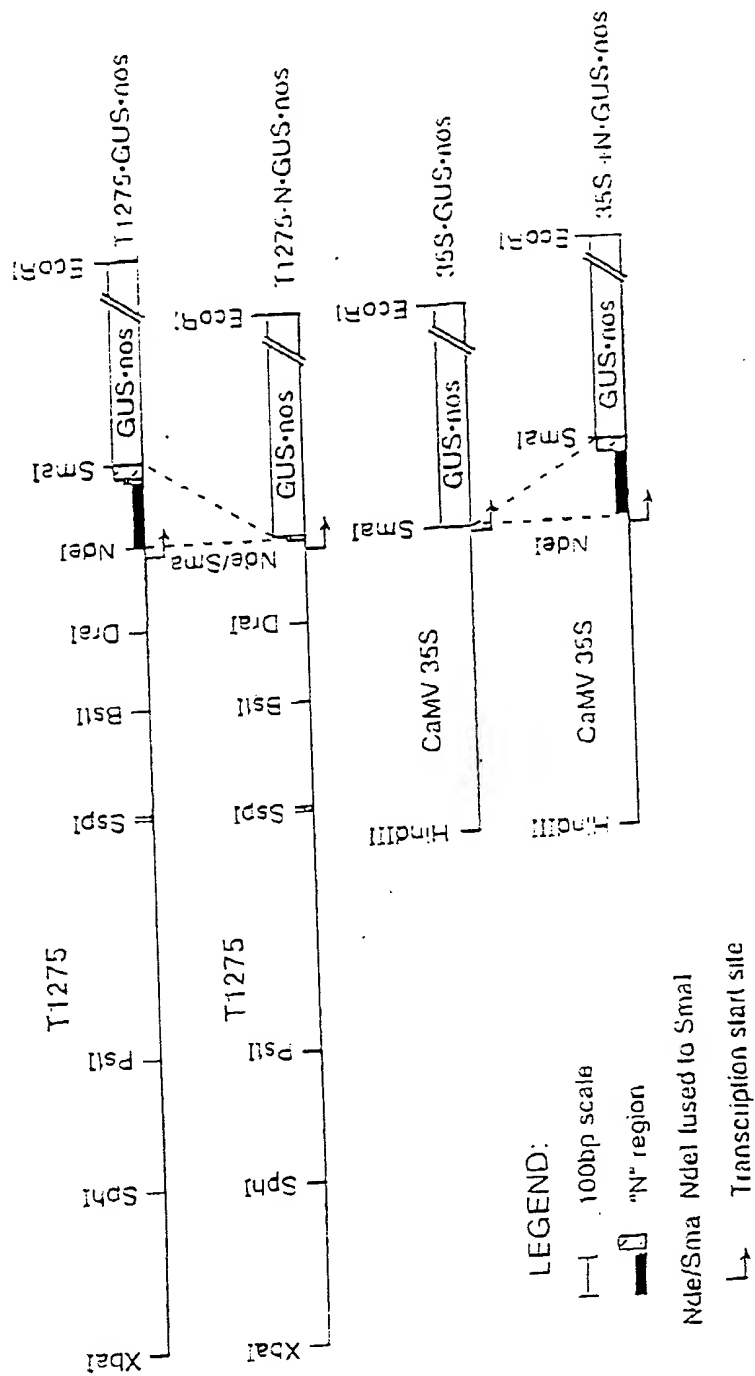


FIGURE 1B

| | 10 | 20 | 30 | 40 | 50 |
|--------|--|----|----|----|----|
| cCUP | 1 ----- | | | | |
| RENT1. | 1 -----A----- ATTGTAAGCG GGATAACAAT | | | | |
| RENT2. | 1 -----AT GTTGTGTGGA ATTGTGAGCG GGATAACAAT | | | | |
| RENT3. | 1 TT----- | | | | |
| RENT5. | 1 -----GGA ATTGTGAGCG G-ATAACAAT | | | | |
| RENT7. | 1 TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG G-ATAACAAT | | | | |

| | 60 | 70 | 80 | 90 | 100 |
|--------|---|----|----|----|-----|
| cCUP | 51 ----- | | | | |
| RENT1. | 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT TTTAATACGA | | | | |
| RENT2. | 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA | | | | |
| RENT3. | 51 ----- | | | | |
| RENT5. | 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATANGA | | | | |
| RENT7. | 51 TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT CT-AATACGA | | | | |

| | 110 | 120 | 130 | 140 | 150 |
|--------|---|-----|-----|-----|-----|
| cCUP | 101 -----TTA TAATTACAAA ATTGATTCTA GTATCTTTAA | | | | |
| RENT1. | 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTAA | | | | |
| RENT2. | 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTAA | | | | |
| RENT3. | 101 -----GATTCTA GTTTTTTTAA | | | | |
| RENT5. | 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTCTA GTATTTTTAA | | | | |
| RENT7. | 101 CTCACTATAG GGAAAGCTTA TAATTACAAA ATTGATTATA GTACTTTTTAA | | | | |

FIGURE 1C1

| | | | | | | |
|--------|-----|------------|------------|------------|------------|------------|
| | 160 | 170 | 180 | 190 | 200 | |
| CCUP | 151 | TTTAATGCTT | ATACATTATT | AATTAATTTA | GTACTTTCAA | TTTGTTTTCA |
| 200 | | | | | | |
| RENT1. | 151 | TTTAATATTT | TTACATTATT | AATTAATTTA | GAAGTTTTAA | TTTTTTTTCA |
| 200 | | | | | | |
| RENT2. | 151 | TTTAATATTT | ATACATTATT | AATTAACTTA | GTACTTTCAA | TTGTTTTTCA |
| 200 | | | | | | |
| RENT3. | 151 | TTTAATATTT | ATACATTATT | AATTAATTTA | GTTCTTTCAA | TTTGTTTTCA |
| 200 | | | | | | |
| RENT5. | 151 | TTTAATATTT | ATACATTATT | AATTAATTTA | GTACTTTCAA | TTTGTTTTCA |
| 200 | | | | | | |
| RENT7. | 151 | TTTAATATTT | ATACATTATT | AATTAATTTA | GCCTTTTCAA | TTTATTTTCA |
| 200 | | | | | | |

| | | | | | | |
|--------|-----|------------|------------|------------|------------|------------|
| | 210 | 220 | 230 | 240 | 250 | |
| CCUP | 201 | GAAATTATTT | TACTATTTTT | TATAAAATAA | AAGGGAGAAA | ATGGCTATTT |
| 250 | | | | | | |
| RENT1. | 201 | GAAATCATTT | TACTATTTTT | -ATPAAAACA | AAAGGGAAAA | GTGGTTATTT |
| 250 | | | | | | |
| RENT2. | 201 | AAAATTATTT | TACTATTTTT | TGTAAAATAA | AAGGGAGAAA | ATGGCTATTT |
| 250 | | | | | | |
| RENT3. | 201 | GAAATTATTT | TACTATTTTT | TATAAAATAA | AAGGGAGAAA | ATGGCTATTT |
| 250 | | | | | | |
| RENT5. | 201 | GAAATCATTT | TACTATGGTT | TATAAAATAA | AAGGGAGAAA | ATGGCTATTT |
| 250 | | | | | | |
| RENT7. | 201 | GAAACCATTT | TACTATTTTT | TATAAAATAA | AAGGGACAAA | ATGGCTATTT |
| 250 | | | | | | |

| | | | | | | |
|--------|-----|------------|------------|------------|------------|------------|
| | 260 | 270 | 280 | 290 | 300 | |
| CCUP | 251 | AAATACTAGC | -CTATTTTAT | TTCAATTTTA | GCCTAAAATC | AG-CCCCAAT |
| 300 | | | | | | |
| RENT1. | 251 | AAATACTAGC | CCTATTTTAT | TTCAATTATA | GCCTAAAATC | AGCCCC-AAT |
| 300 | | | | | | |
| RENT2. | 251 | AAATACTAGC | CCTATTTTAT | TTCAATTTTA | GCCTAAAATC | AGCCCCCAAT |
| 300 | | | | | | |
| RENT3. | 251 | AAATACCAGC | CCTATTTTAT | TTCAATTTTA | ACCTAAAATC | AGCCCC-AGT |
| 300 | | | | | | |
| RENT5. | 251 | AAATACTAGC | CCTATTTTAT | TTCAATTTTA | GCCTAAAATC | AGCCCC-AAT |
| 300 | | | | | | |
| RENT7. | 251 | AAATACCAAC | ACTATTTTAT | TTCAATTTTA | GCCTAAAATC | AAACCC-AAT |
| 300 | | | | | | |

FIGURE 1C2

310 320 330 340 350
 301 TAGCCCCAAT TTCAAATTCA AATGGTCCAG CCCAATTCCT AAA-TAACCC
 301 TAACCCCAAT TCCAAATTCA AACGGGCCAG CCCAATTCCT AAAATGACCC
 301 TAACCCCAAT TTCAAATTCA AATGGGACAG CCCAATTCCT AAAATAACCC
 301 TAGCCCC--- -----A AACGGGCCAT CCCAATTCCT AAAATAACTC
 301 TAACCCCTAT TTCARATTCA AACGGGCTAG CCCAGTTCCT AAAATAACCC
 301 TAACCCC--- -----A AACGGGCCAG CCCAATTCCT AAAACAACCC

360 370 380 390 400
 351 ACCCCTAACC C----- ----GCCCCG TTTCCCCTTT TGATCCAGGC
 351 GCTCCTAACC CGCTTTTCCA ACCCGCCCCG TTTCCCCTTT TGATCCAGGC
 351 GCCCCTAACC CTCTTATCCA ACCCACCAGA TTTCCCCTTT TGATCCAGGT
 351 GCCCCTAACC CGCTTATCCA ACCCGCCCCG TTTCC-CTTT TGATCCAGGC
 351 TCCCCTAACC CGCTTATCCA ACCCGCCCTG TTTCCCCTTT TGATCCAGGC
 351 GCCCCTAACC CGCTTATCCA ACCCGCCCCG TTTCTCTTT TGATCCAGGC

410 420 430 440 450
 401 CGTTGATCAT TTTGATCAAC GCCCAGAATT TCCCCTTTTC CTTTTTTAAT
 401 TGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTTC --TTTTTAAT
 401 TGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCTTTTC TGTTTTTAAT
 401 CGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCTTTTC -TTTTTTAAT
 401 CGTTGATCAT TTTGATCAAC GACCAAAATT TCCCCTTT-C CTTTTTTAAT
 401 CGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTTC -TTTTTTCAT

FIGURE 1C3

| | 460 | 470 | 480 | 490 | 500 | |
|--------|-----|------------|------------|------------|------------|------------|
| LCUP | 451 | TCCCAAACAC | C-CCTAACTC | TATCCCATTT | CTCACCAACC | GCCACATATG |
| 500 | | | | | | |
| RENT1. | 451 | TCCCAAACAC | CCCCCAACCT | TATCCCGTTT | CTCACCAACC | GCCAGATCT- |
| 500 | | | | | | |
| RENT2. | 451 | TCCCAAACAC | CCCCCAACCC | TATCCCATTT | CTCACCAACC | GCCAGATCT- |
| 500 | | | | | | |
| RENT3. | 451 | TCCCAAACAC | CGCC-AAACC | TATCCCATTT | CTCACCAACC | GCCAGATCTA |
| 500 | | | | | | |
| RENT5. | 451 | TCCCAAACAC | CCCC-AAACC | TATCCCATTT | CTCACCAACC | GCCAGATCT- |
| 500 | | | | | | |
| RENT7. | 451 | TCCCAAACAC | CCCC-AAACC | TATCCCATTT | CTCACCAACC | GCCAGATCTA |
| 500 | | | | | | |

| | 510 | 520 | 530 | 540 | 550 | |
|--------|-----|------------|------------|------------|------------|------------|
| LCUP | 501 | AATCCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |
| RENT1. | 501 | -ATCCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |
| RENT2. | 501 | -ATCCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |
| RENT3. | 501 | T--CCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |
| RENT5. | 501 | -ATCCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |
| RENT7. | 501 | T--CCTCTTA | TCTCTCAAAC | TCTCTCGAAC | CTTCCCCTAA | CCCTAGCAGC |
| 550 | | | | | | |

| | 560 | 570 | 580 | 590 | 600 | |
|--------|-----|------------|------------|------------|------------|------------|
| LCUP | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GAATACATGG | CTTCTCAAGC |
| 600 | | | | | | |
| RENT1. | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GCCACCATGG | CCTCTAGAG- |
| 600 | | | | | | |
| RENT2. | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GCCACCATGG | CCTCTAGAG- |
| 600 | | | | | | |
| RENT3. | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GCCACCATGG | CCTCTAGAGG |
| 600 | | | | | | |
| RENT5. | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GCCACCATGG | CCTCTAGAG- |
| 600 | | | | | | |
| RENT7. | 551 | CTCTCATCAT | CCTCACCTCA | AAACCCACCG | GCCACCATGG | CCTCTAGAGG |
| 600 | | | | | | |

FIGURE 1C4

| | 610 | 620 | 630 | 640 | 650 |
|--------|-----|------------|----------------|------------------------|-----------------------|
| ECUP | 601 | CGTGGAACC | TTATACTCAC | CTCCCTTTGC | TCTTACAGTA CTC-GGCCGT |
| 650 | | | | | |
| RENT1. | 601 | ---GATCCCC | GGGTGGTCAG | TCCCTTATG- --TTAC---- | -----GT |
| 650 | | | | | |
| RENT2. | 601 | ----GA---- | -----TCCCCG--- | -----GGTG | GTC-AGTCCC |
| 650 | | | | | |
| RENT3. | 601 | ATC----CCC | GGGTGGTCAG | TCCCTTATGT NA----- | CG NCCTAAATGN |
| 650 | | | | | |
| RENT5. | 601 | ---GATCCCC | GGGTGGTCAG | TCCCTTATG- --TTACG---- | ----- |
| 650 | | | | | |
| RENT7. | 601 | ATC----CCC | GGGTGGTCAG | TCCCTTATGT TA----- | CG TCCTN----- |
| 650 | | | | | |

| | 660 | 670 | 680 | 690 | 700 |
|--------|-----|------------|------------|-----|-----|
| ECUP | 651 | CGACCGCGGT | ACCCGGG... | | |
| 700 | | | | | |
| RENT1. | 651 | C----- | --CTNAA... | | |
| 700 | | | | | |
| RENT2. | 651 | TTAT-GTG-- | ---CGTC... | | |
| 700 | | | | | |
| RENT3. | 651 | CCGNCCTGNN | NNNNN-C... | | |
| 700 | | | | | |
| RENT5. | 651 | ----- | TCC----- | | |
| 700 | | | | | |
| RENT7. | 651 | ----- | | | |
| 700 | | | | | |

FIGURE 1C5

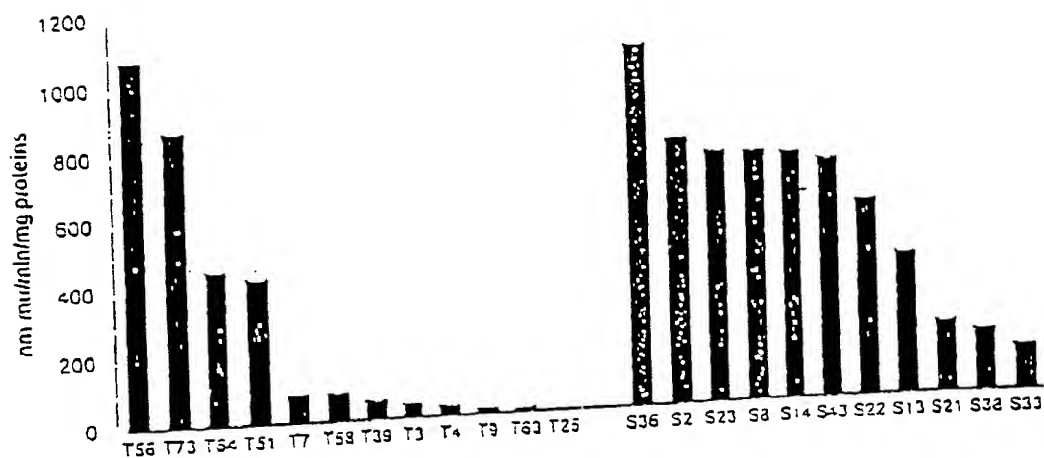


FIGURE 2A

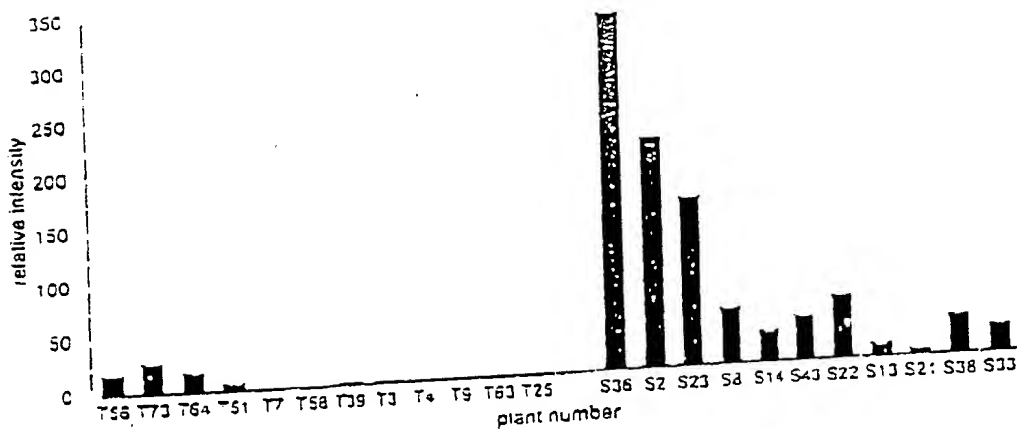


FIGURE 2B

SRI T56 T64 T58 T9 S36 S14 S13 S33 E.c.

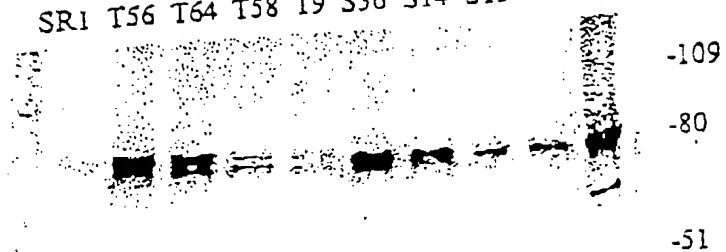


FIGURE 2C

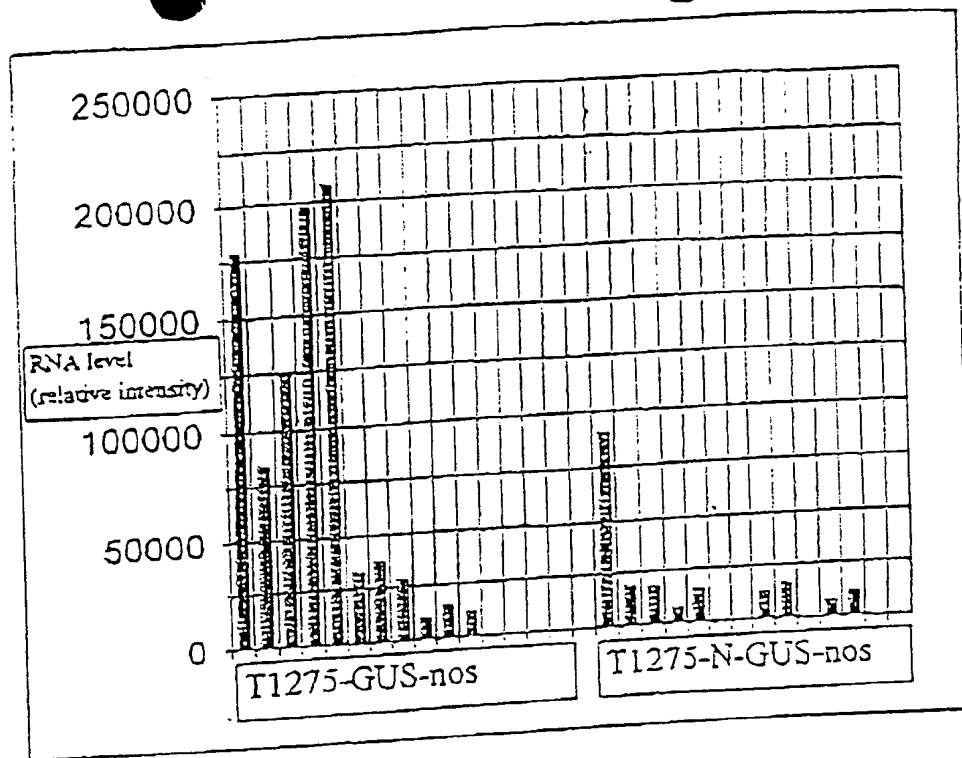


FIGURE 3A

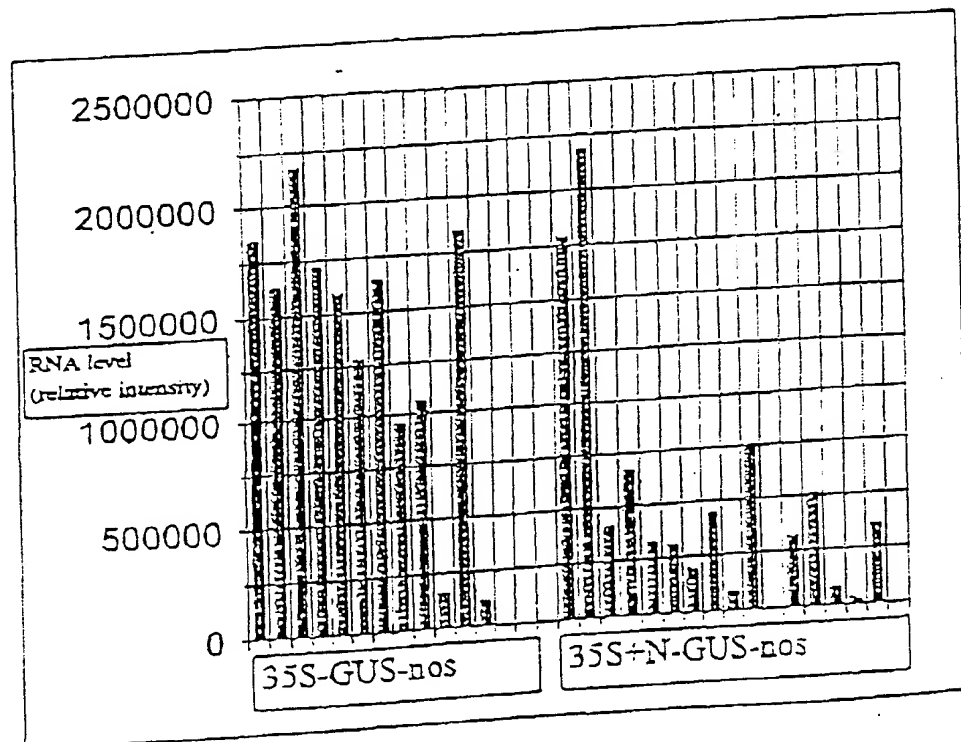


FIGURE 3B

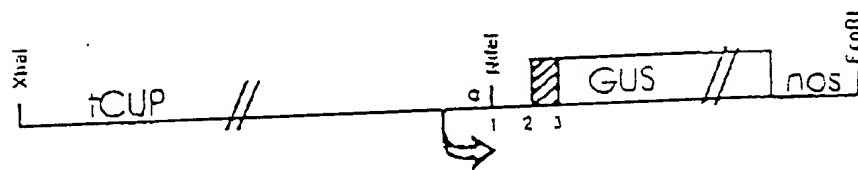
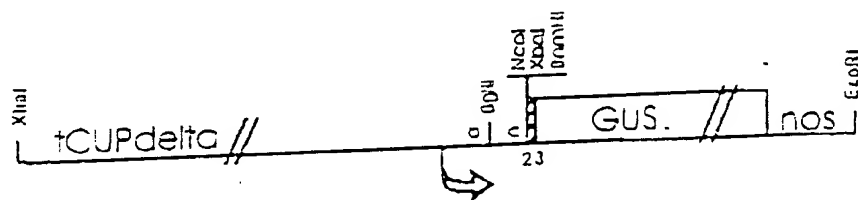


FIGURE 4A



- a NotI position -30 $\overline{\text{ACA TAT GAA}}$
 b BglII position +25 $\overline{\text{ACA GAT CT}}$
 c NotI position 0 $\overline{\text{CC ACC ATG GCC TCT AGA GGA TCC CCG GGT GGT CAG TCC CTT ATG}}$
 tCUP initiation site $\overline{\text{GAA TAC ATG G}}$ / ...tCUP leader ... / $\overline{\text{CCG GGT GGT CAG TCC CTT ATG}}$
 Kazik consensus $\overline{\text{CC ACC ATG G}}$

FIGURE 4B

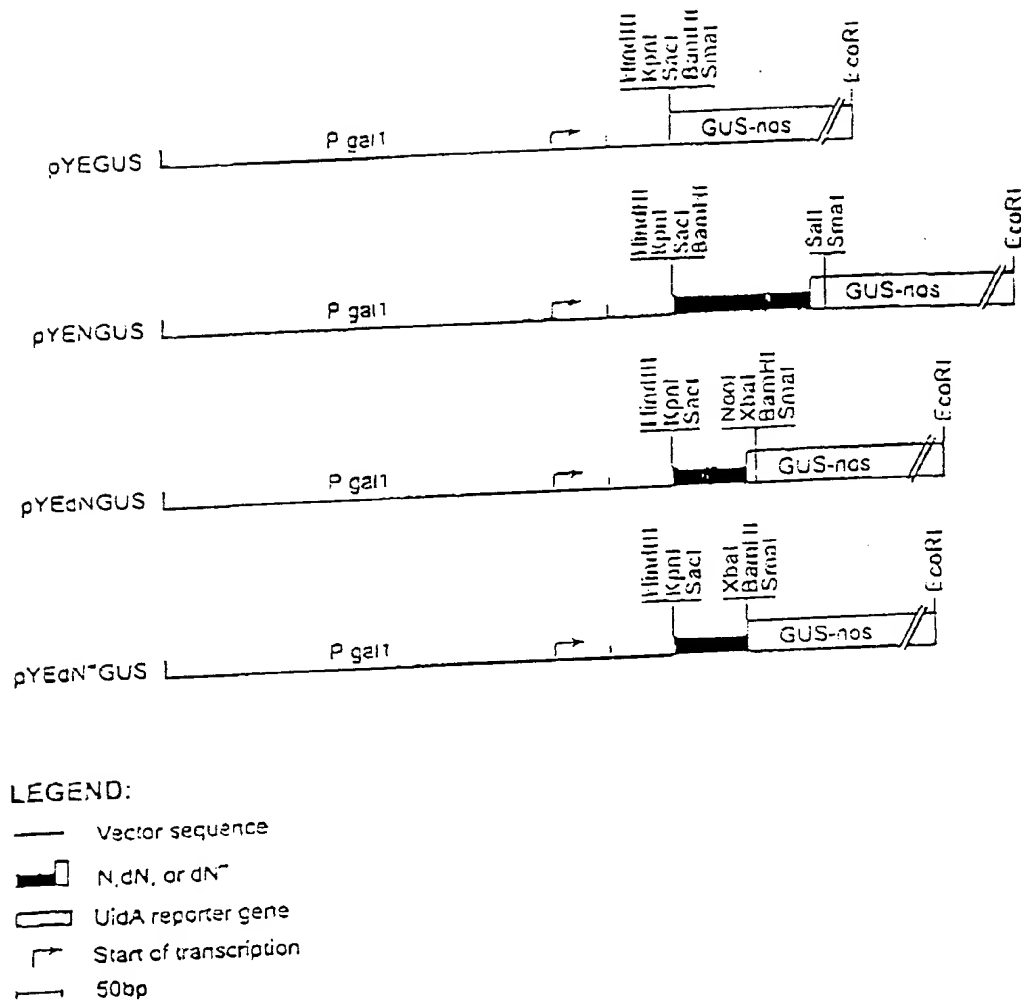


FIGURE 5A

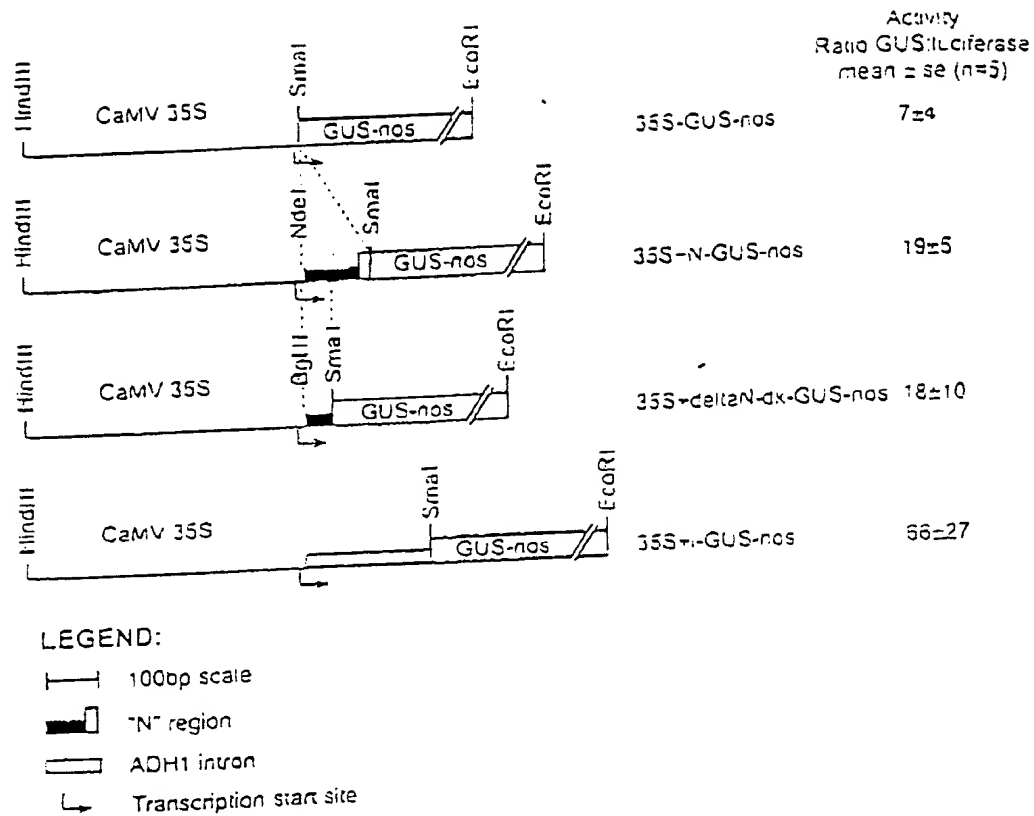


FIGURE 5B

White Spruce Callus Transient Analysis 14/12/99

| Construct | GUS:LUC Ratio (approx.) |
|--------------|-------------------------|
| i-CUP | 25 |
| i-CUP+header | 5 |
| 35S | 30 |
| 35S+header | 240 |

FIGURE 5C

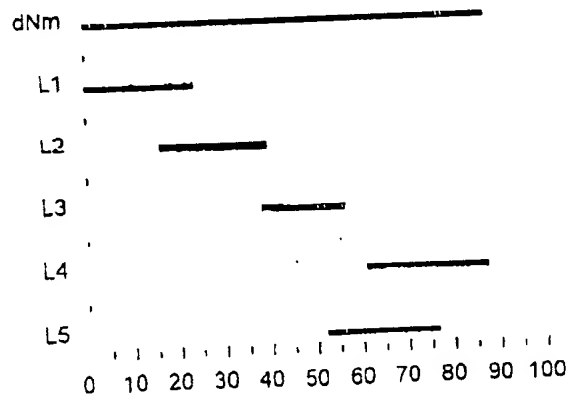


FIGURE 6A

Linker 1: GGATCTATCCTCTTATCTCTCAA
 Linker 2: ATCTCTCAAACCTCTCTCGAACCTT
 Linker 3: TTCCCTAACCCCTAGCAG
 Linker 4: ATCATCCTCACCTCAAAACCCACC
 Linker 5: AGCCTCTCATCATCCTCACCTCAA

FIGURE 6B

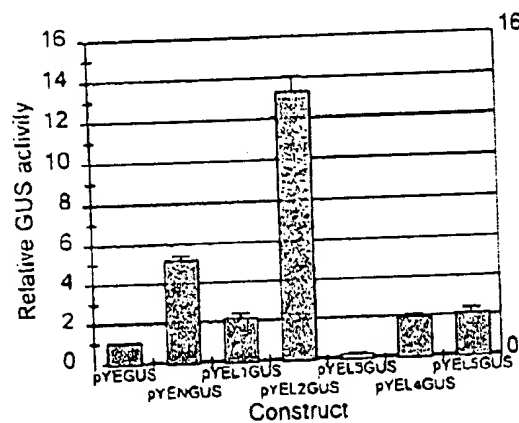


FIGURE 6C

L2 AUCUCUCAAA**ACUCUCUC**GAACCUU
 L2C AUCUCUCAAAACUCUCU
 L2R ACUCUCUCGAACCUU

FIGURE 6D

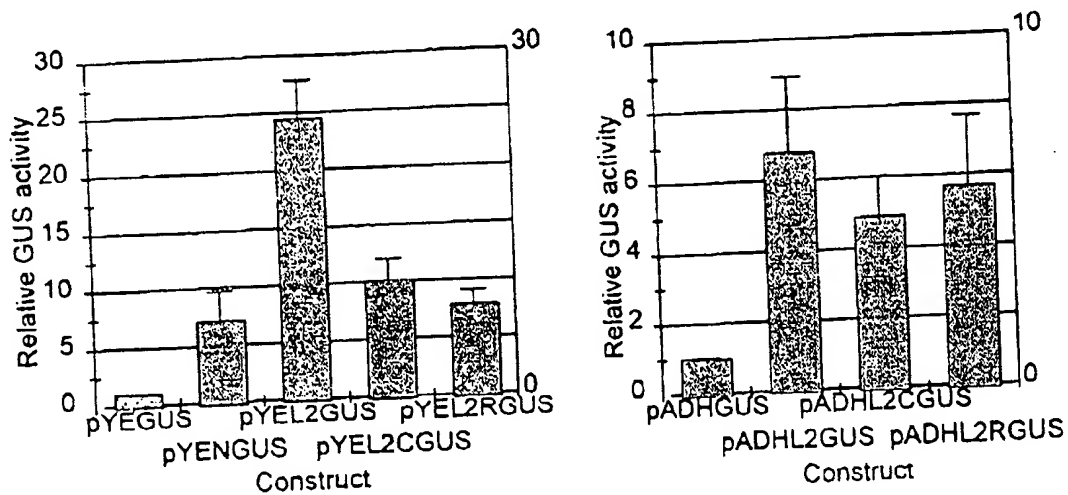


FIGURE 6E

| | | | | | | | | | |
|-------|---|-----|-----|-----|-----|-----|-----|-----|----|
| L2 | A | TCT | CTC | AAA | CTC | TCT | CGA | ACC | TT |
| SCAN1 | a | AGA | ctc | aaa | ctc | tct | cga | acc | tt |
| SCAN2 | a | tct | GAG | aaa | ctc | tct | cga | acc | tt |
| SCAN3 | a | tct | ctc | GGG | ctc | tct | cga | acc | tt |
| SCAN4 | a | tct | ctc | aaa | GAG | tct | cga | acc | tt |
| SCAN5 | a | tct | ctc | aaa | ctc | AGA | cga | acc | tt |
| SCAN6 | a | tct | ctc | aaa | ctc | tct | GCT | acc | tt |
| SCAN7 | a | tct | ctc | aaa | ctc | tct | cga | GAG | tt |

FIGURE 6F

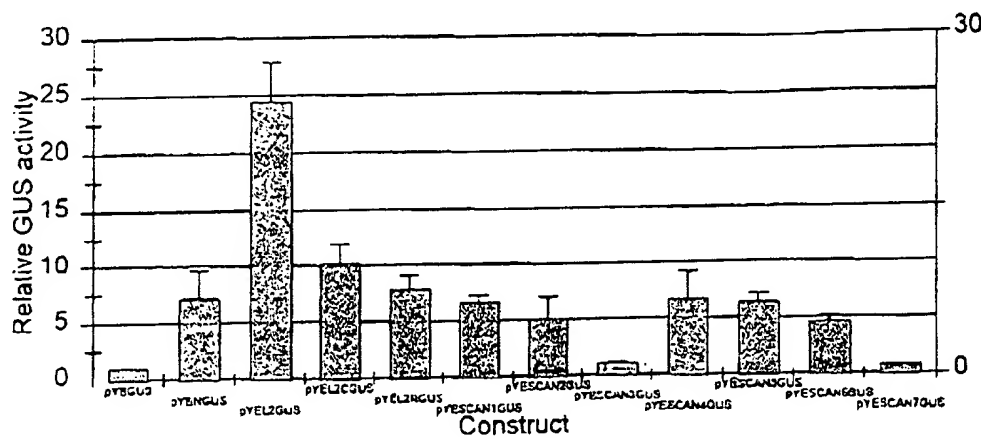


FIGURE 6G

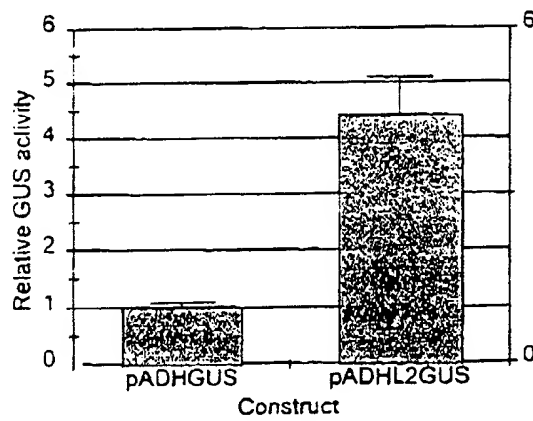
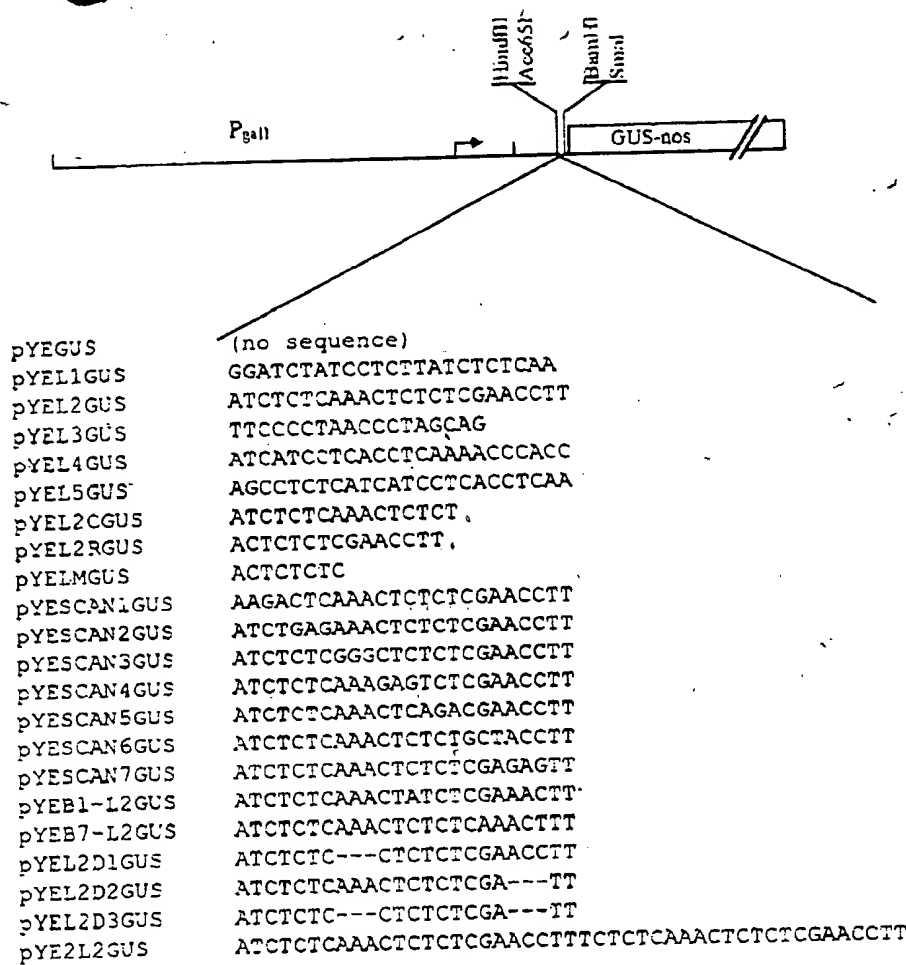


FIGURE 6H

004400-422400



LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 6I

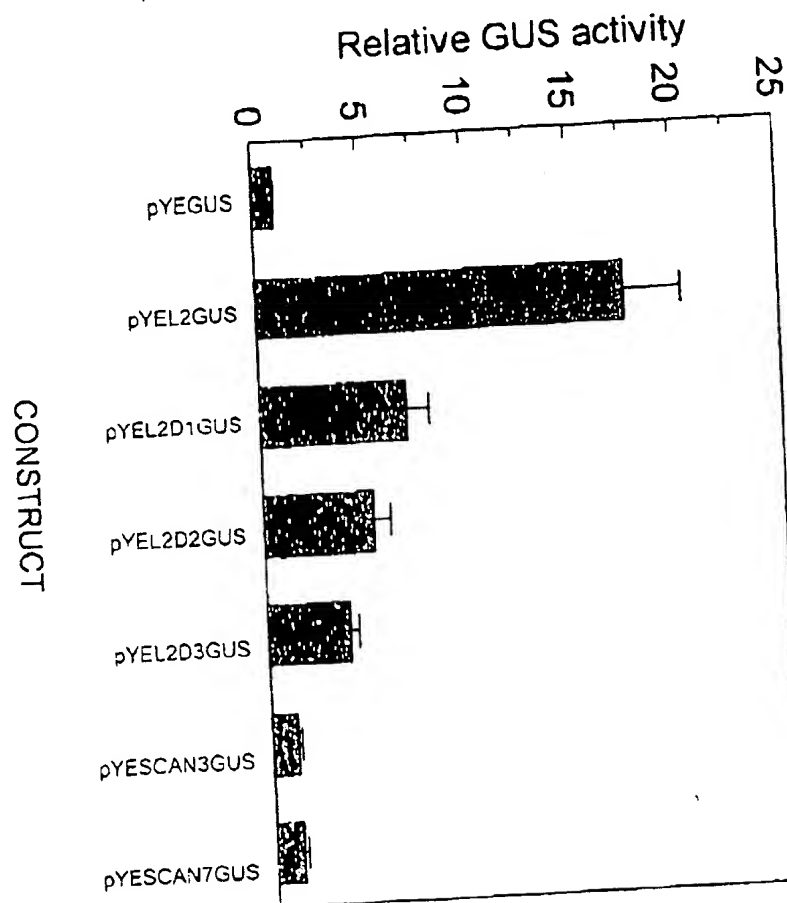


FIGURE 6J

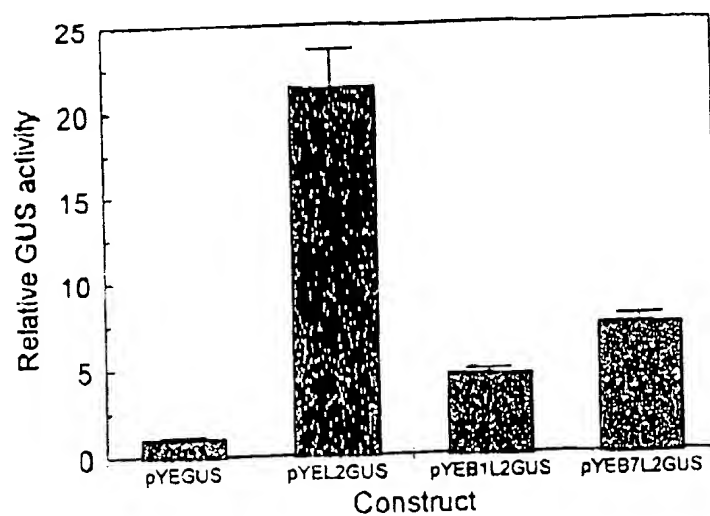


FIGURE 6K

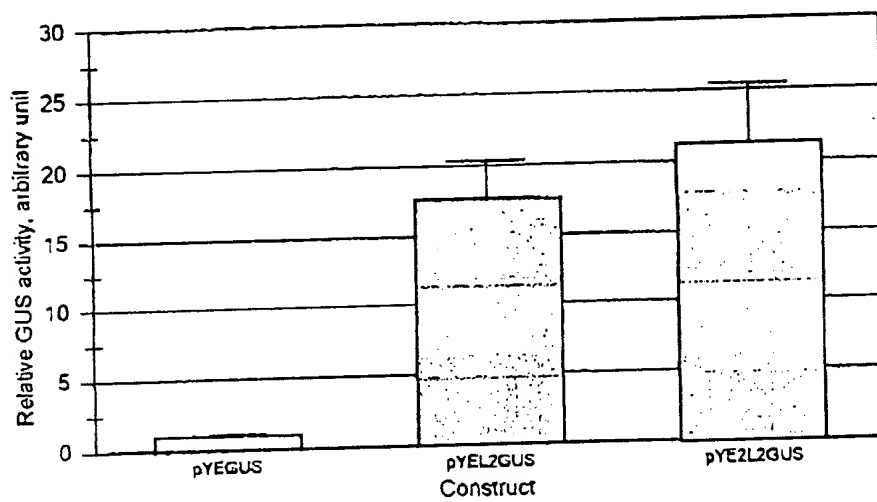
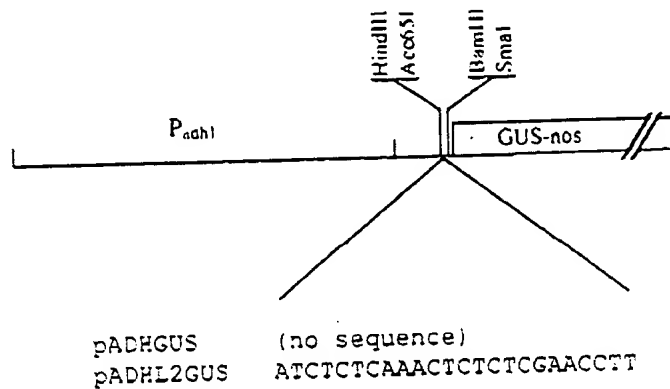


FIGURE 6L



LEGEND:

- Vector sequence
- ▭ GUS reporter gene

FIGURE 6M

Figure 6N shows the GUS reporter gene construct. The construct is a linear DNA molecule containing the GUS reporter gene (GUS-nos) and the P_{gusII} promoter. The construct is flanked by Acc65I restriction sites. The GUS reporter gene is transcribed from the P_{gusII} promoter. The construct is shown in a schematic diagram and a sequence alignment.

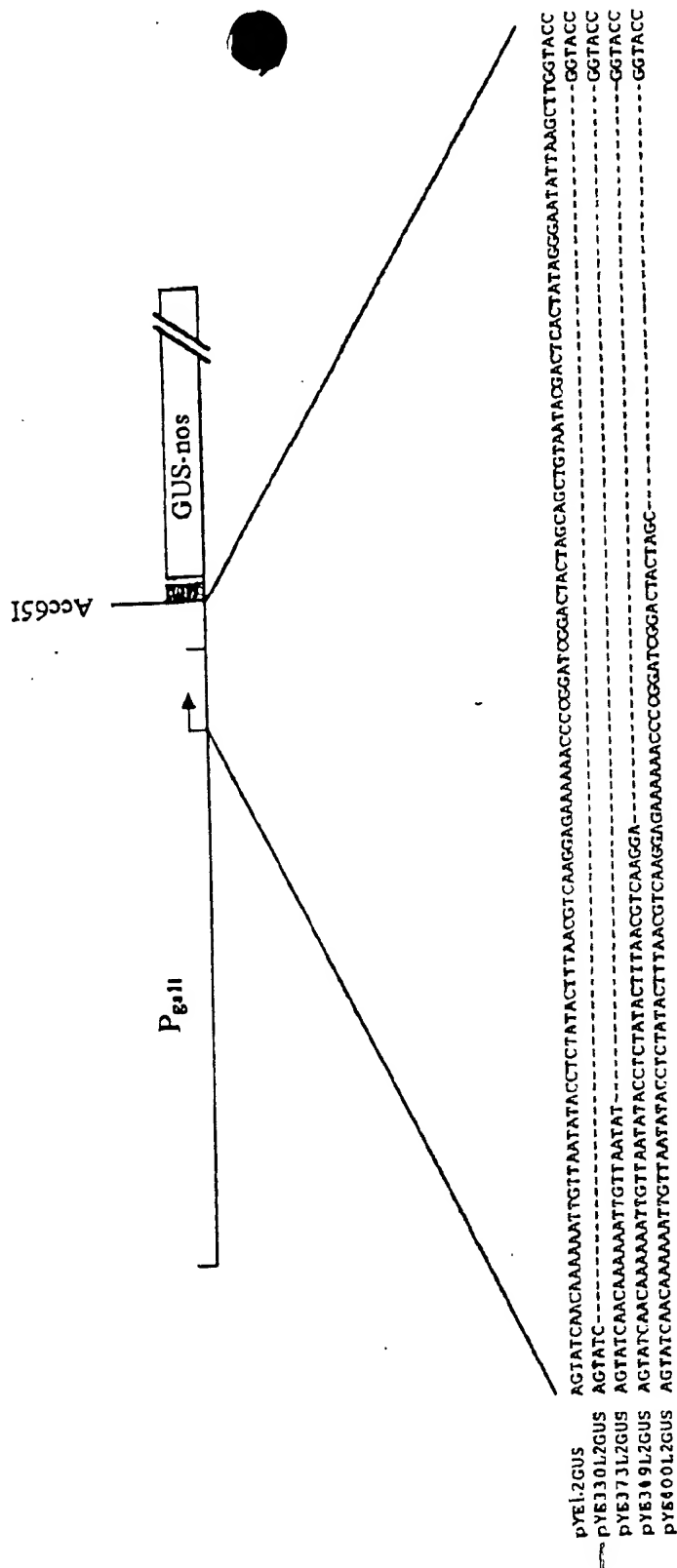


FIGURE 6N

LEGEND:

- Vector sequence
- GUS reporter gene
- ↑ Start of transcription
- L2

Analysis of GUS activity in yeast expression system

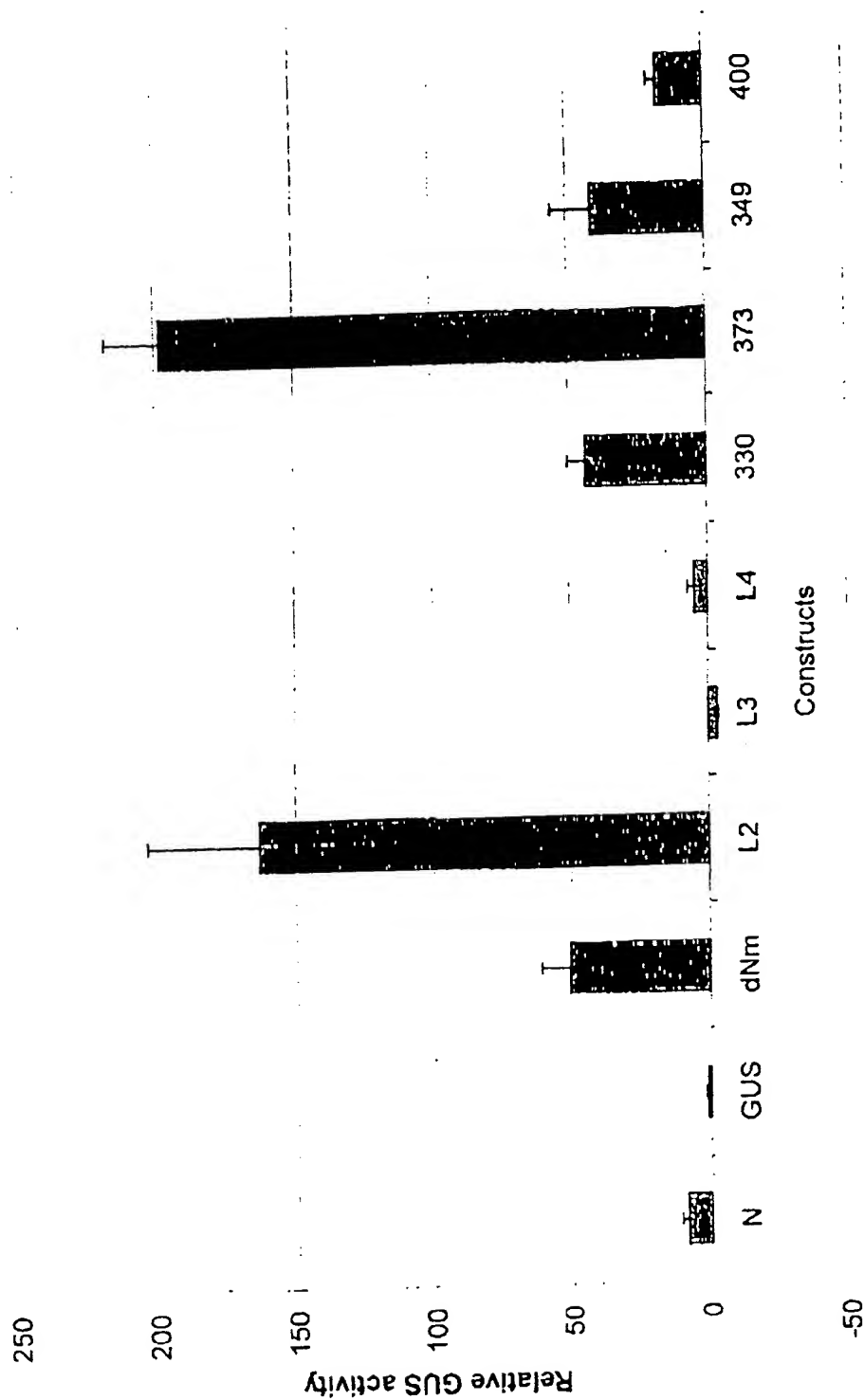


FIGURE 60

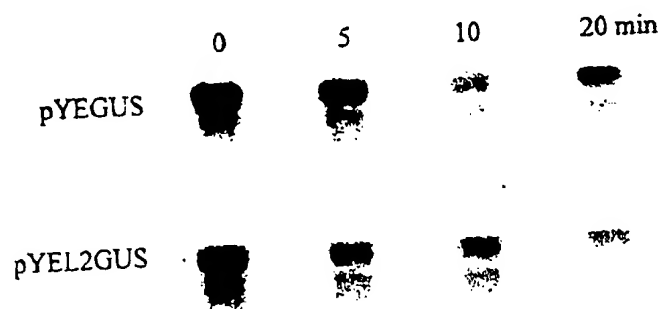


FIGURE 6P.1

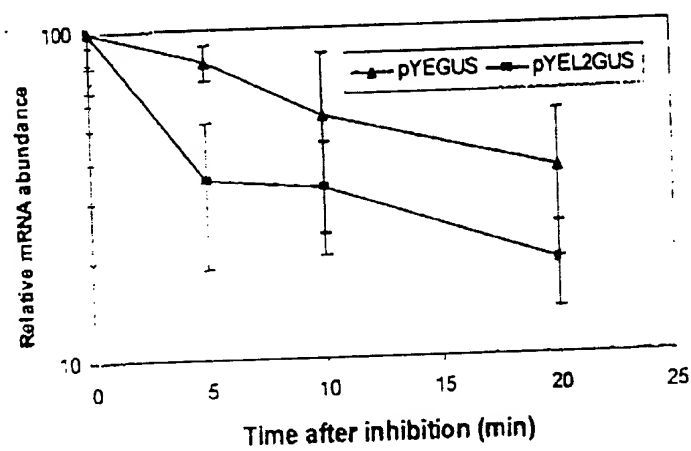


FIGURE 6P.2

Enhanced tCUP Versions 1-3

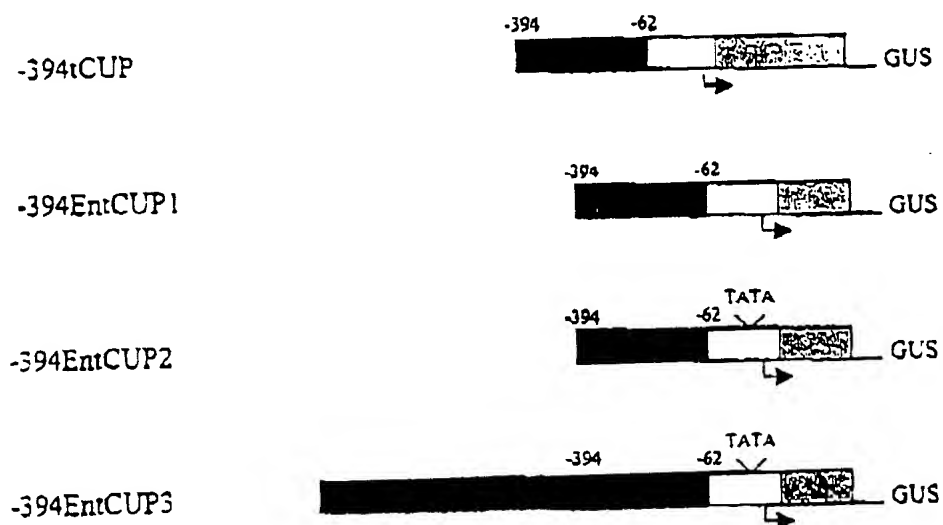
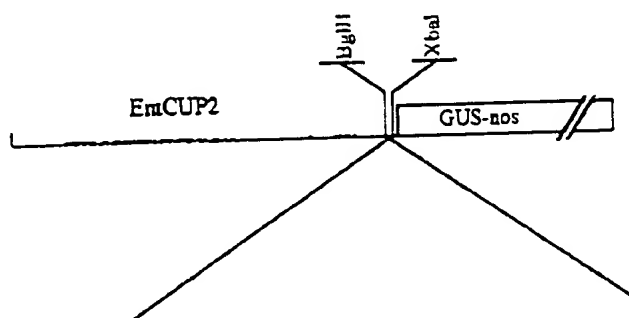


FIGURE 7A.1



pUCtCUP2(-N)GUS
 pUCtCUP2L1GUS
 pUCtCUP2L2GUS
 pUCtCUP2L3GUS
 pUCtCUP2L4GUS
 pUCtCUP2L5GUS
 pUCtCUP2SCAN3GUS
 pUCtCUP2SCAN7GUS
 pUCtCUP2-2XL2GUS

(no sequence)
 GGATCTATCCTCTTATCTCTCAA
 ATCTCTCAAACCTCTCTCGAACCTT
 TTCCCCTAACCCCTAGCAG
 ATCATCCTCACCTCAAAACCCACC
 AGCCTCTCATCATCCTCACCTCAA
 ATCTCTCGGGCTCTCTCGAACCTT
 ATCTCTCAAACCTCTCTCGAGAGTT
 ATCTCTCAAACCTCTCTCGAACCTTCTCTCAAACCTCTCTCGAACCTT

FIGURE 7A.2

Evaluation of tCUP leader elements, L1, L2, L3, L4, and L5 on transient GUS gene expression in white spruce callus

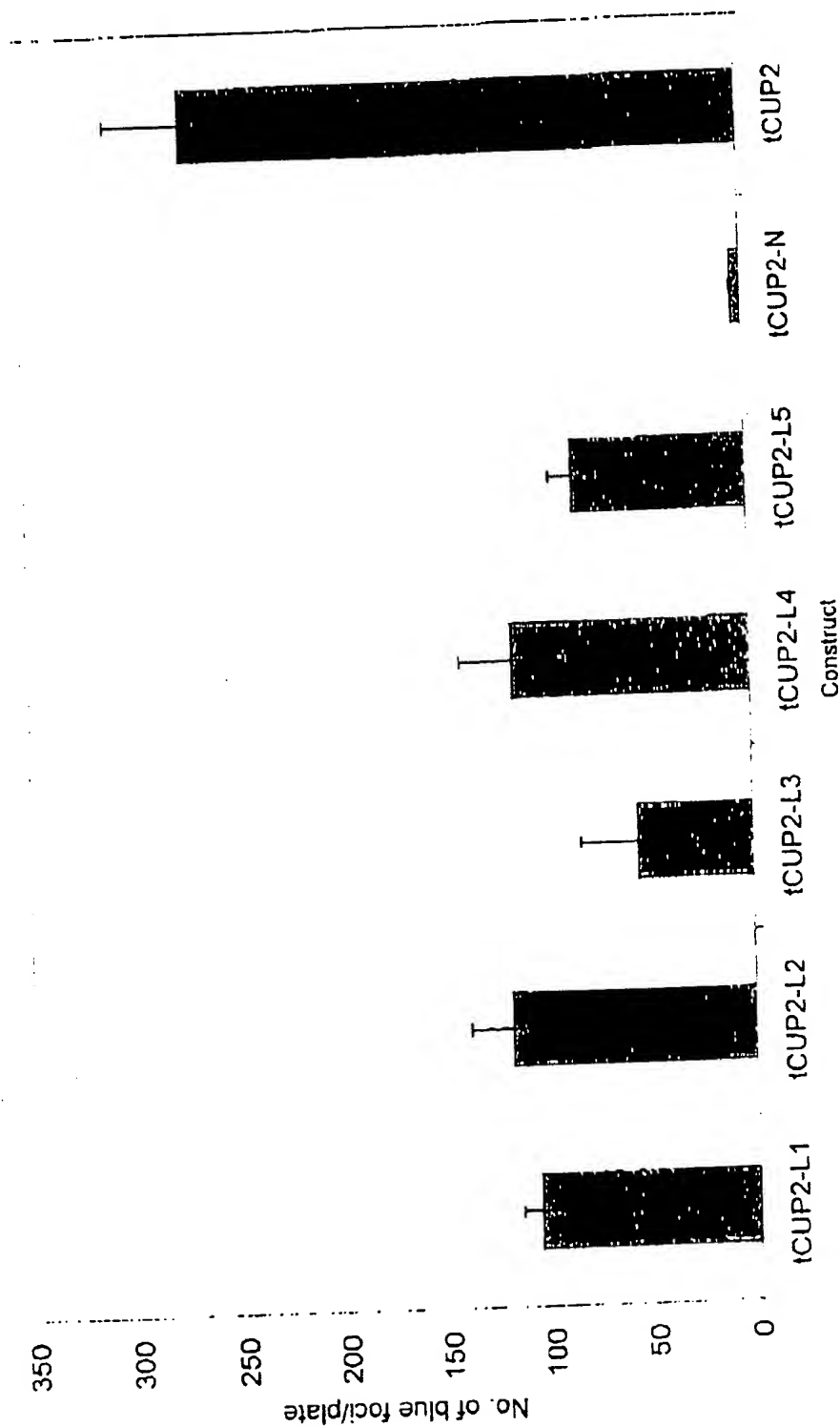
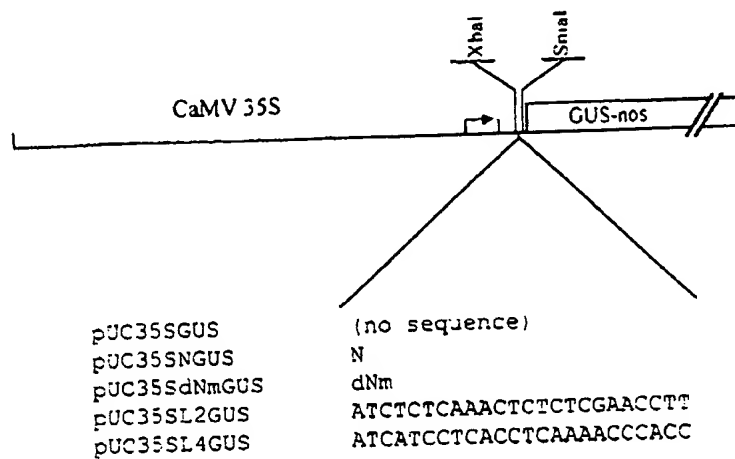


FIGURE 7D



LEGEND:




-  Vector sequence
-  GUS reporter gene
-  Start of transcription

FIGURE 8A

Stable Transformation of *Arabidopsis* with GUS enhanced by
L-series constructs and the 35S promoter

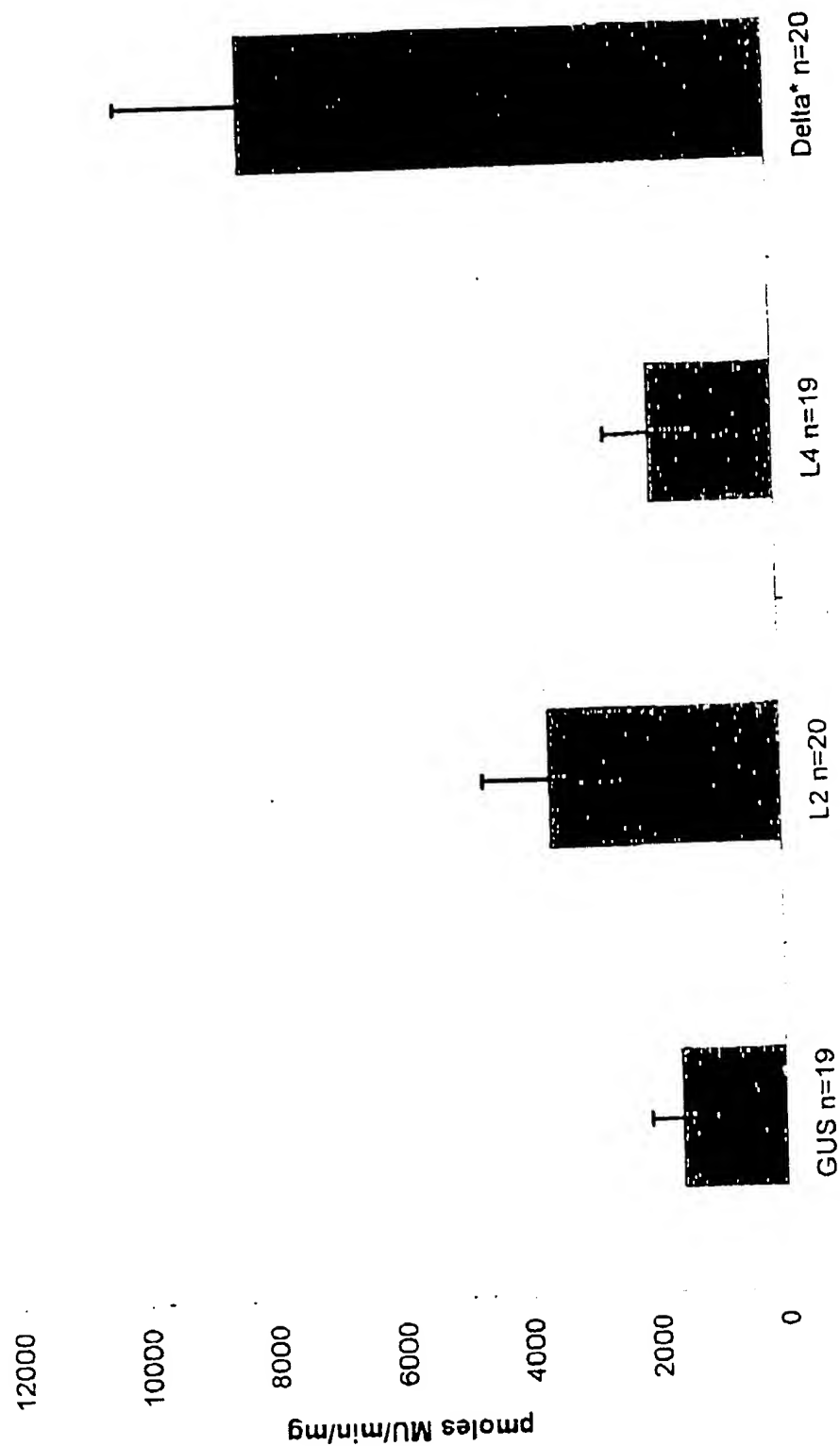


FIGURE 8B

4513 563 0859

Effects of L2 and L4 on 35S Tobacco Transient Assay

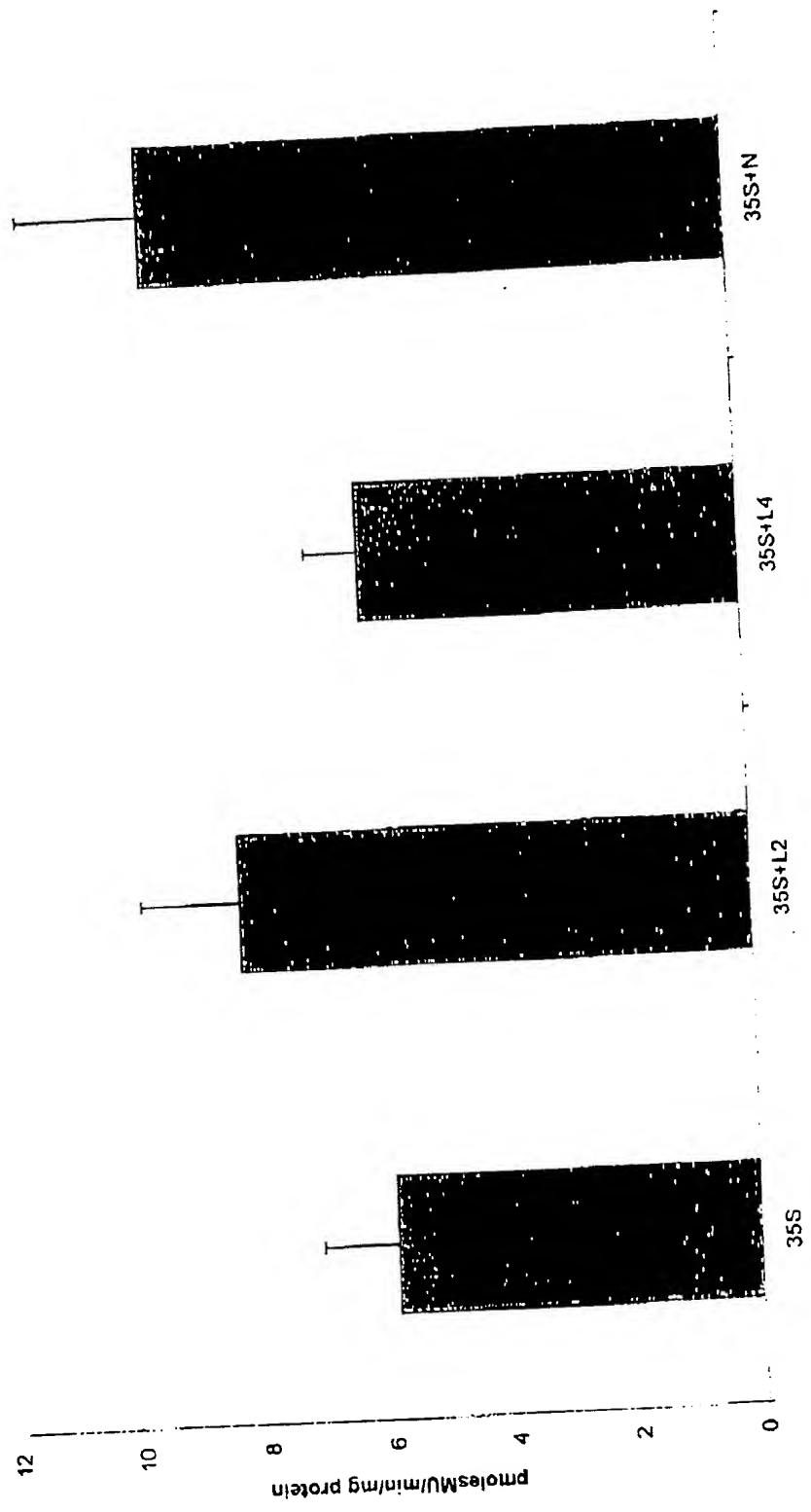


FIGURE 8D

Evaluation of the expression of ICUP leader and the elements, L2 and L4, with a heterologous promoter (35S) in a transient GUS gene expression in alfalfa suspension culture

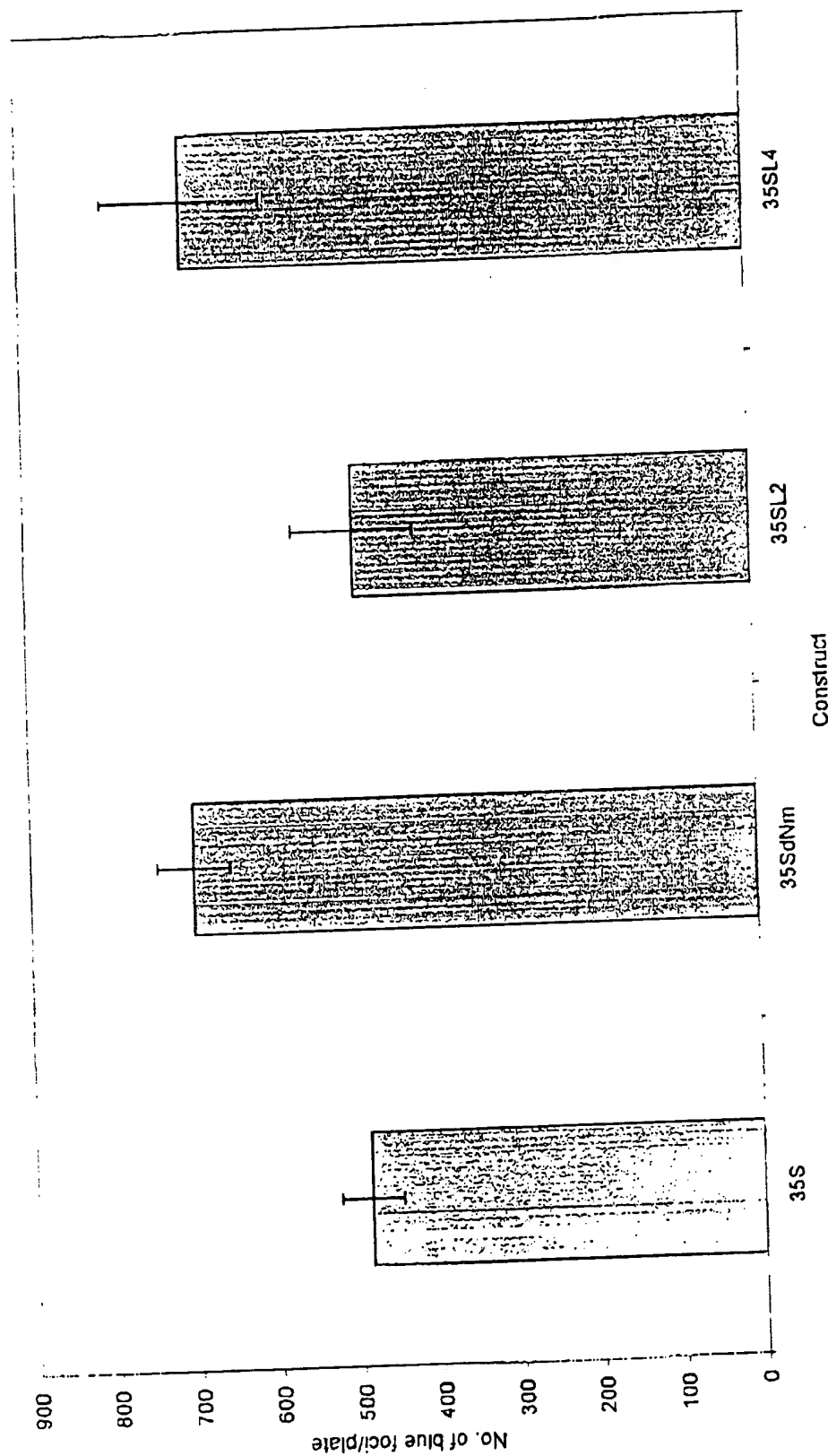
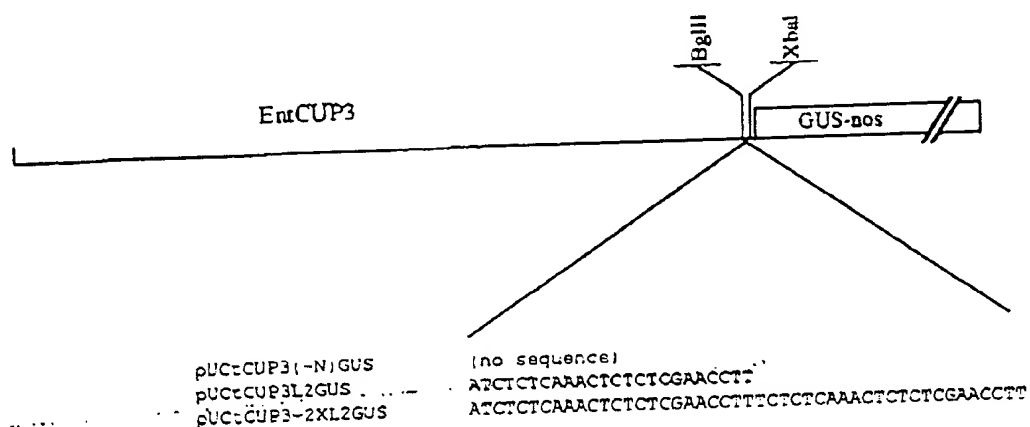


FIGURE 8E

| Construct | n | Avg No. of Foci/plate (approx.) | Significance |
|-----------|---|---------------------------------|--------------|
| GUS | 6 | 200 | +++ |
| L2 | 6 | 150 | +++ |
| L4 | 5 | 270 | ++++ |
| Della | 6 | 420 | +++++ |

FIGURE 8F



LEGEND:

- Vector sequence
- GUS reporter gene

FIGURE 9A

GUS Expression of L2 Scan mutations and enh-tCUP2 in Tobacco Transient Assay

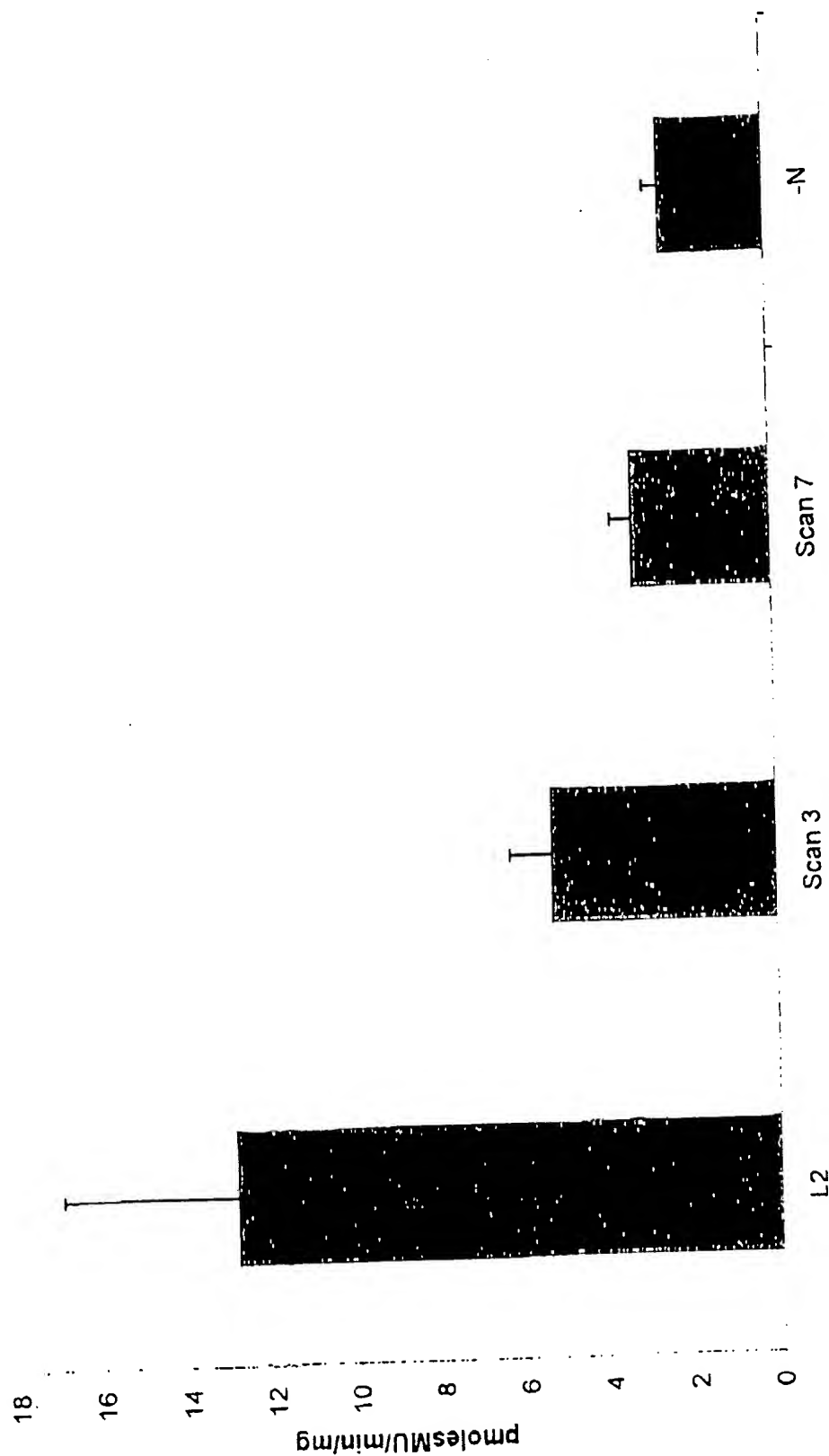


FIGURE 9B

Stable Transformation of *Arabidopsis* with GUS enhanced by L2 and 2XL2 constructs and the enh-tCUP2 and enh-tCUP3 promoter

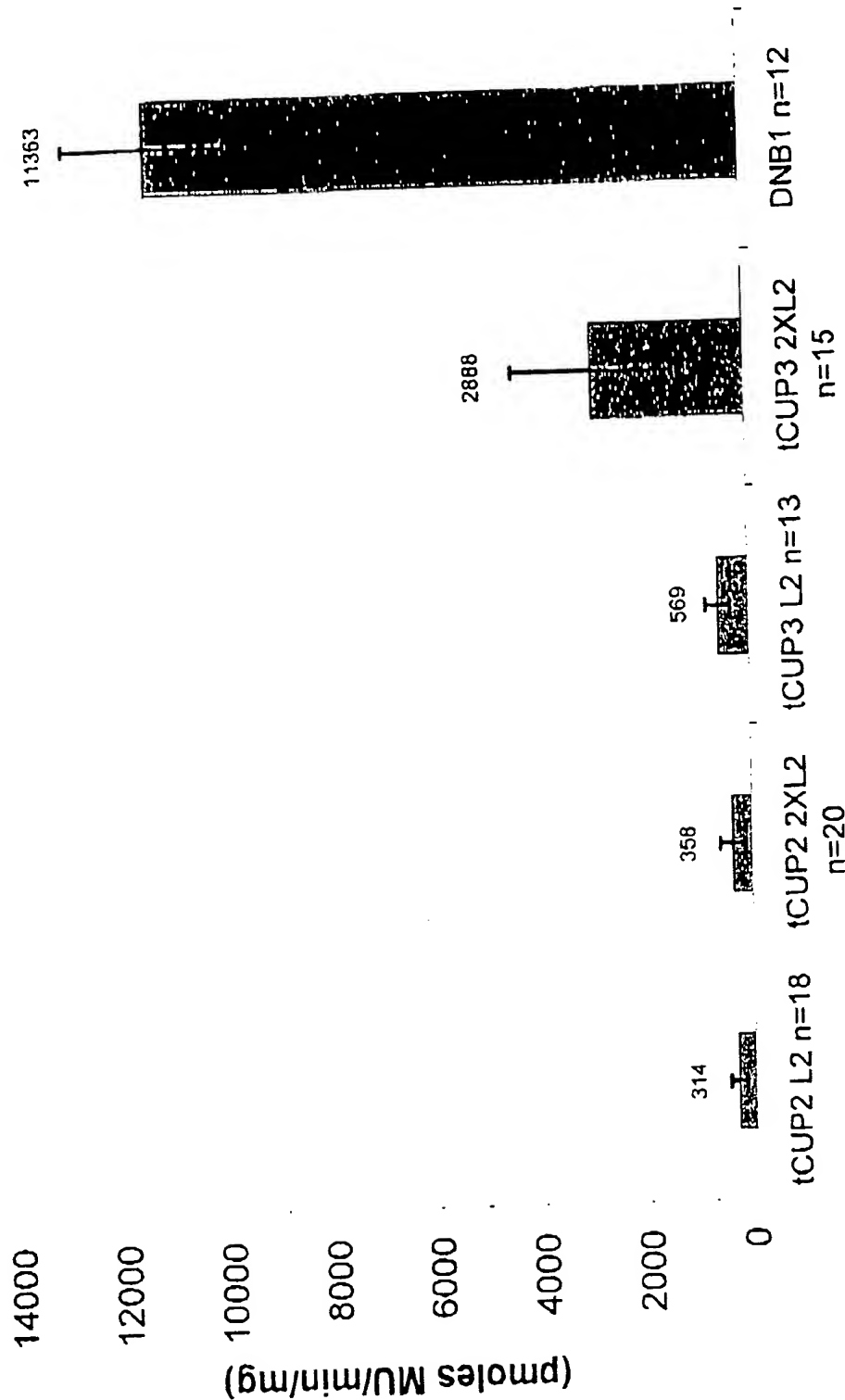
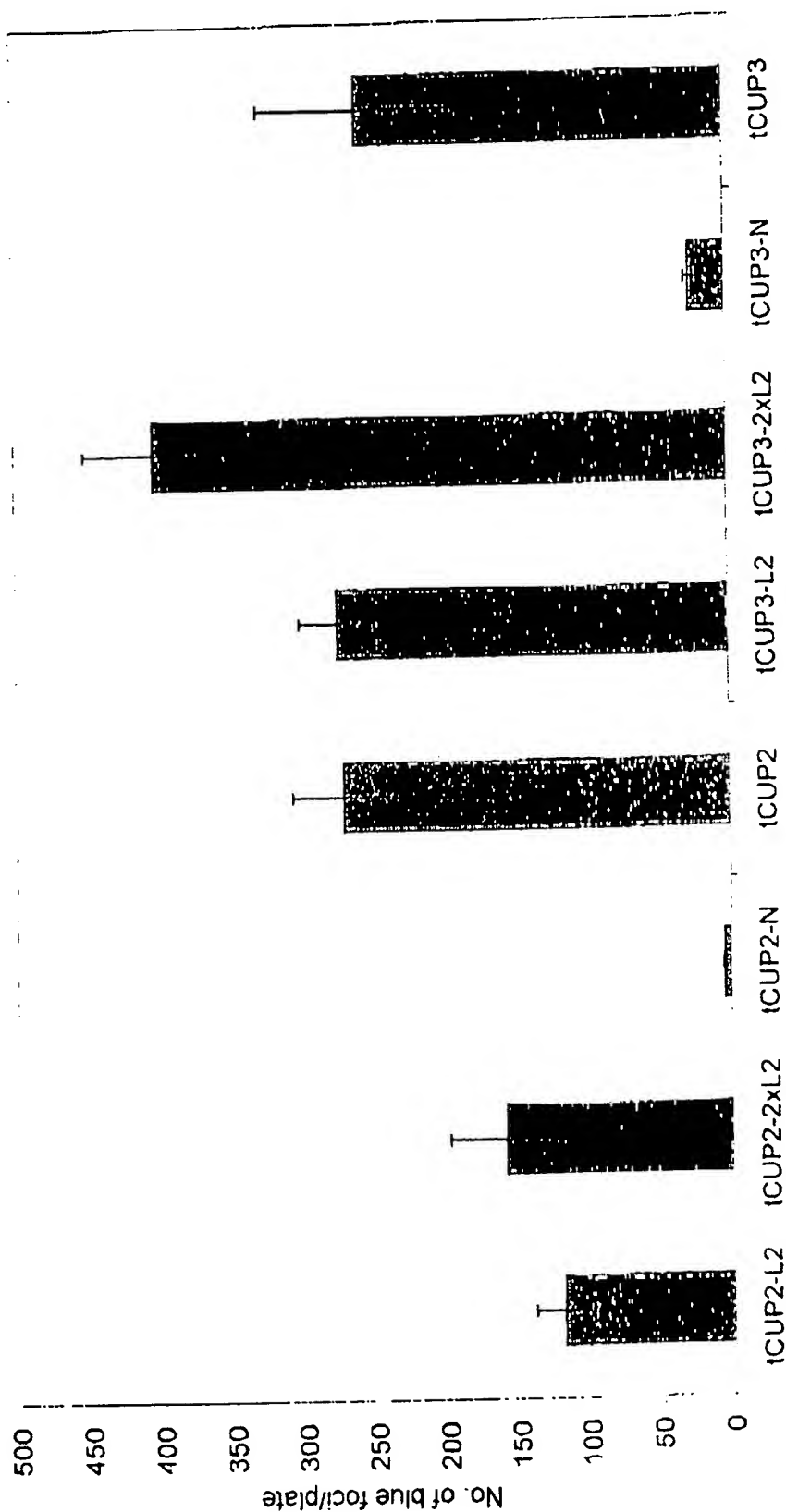


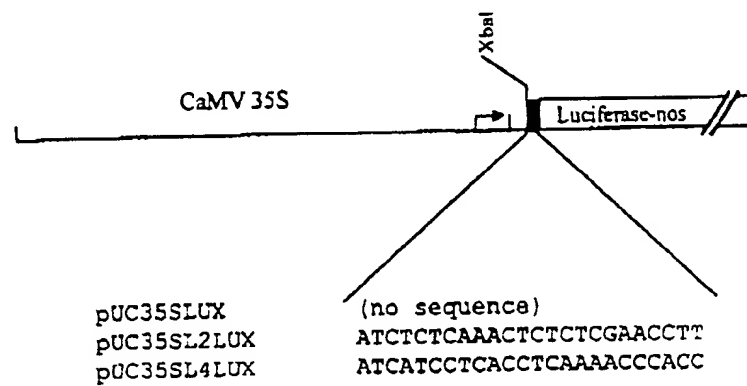
FIGURE 9C

Evaluation of tCUP leader element, L2, on transient GUS gene expression in white spruce callus



Construct

FIGURE 9F



LEGEND:

- Vector sequence
- ▭ Luciferase reporter gene
- ▬ L2 or L4
- Start of transcription

FIGURE 10A

Analysis of L2 and L4 in E. Coli using a luciferase reporter system

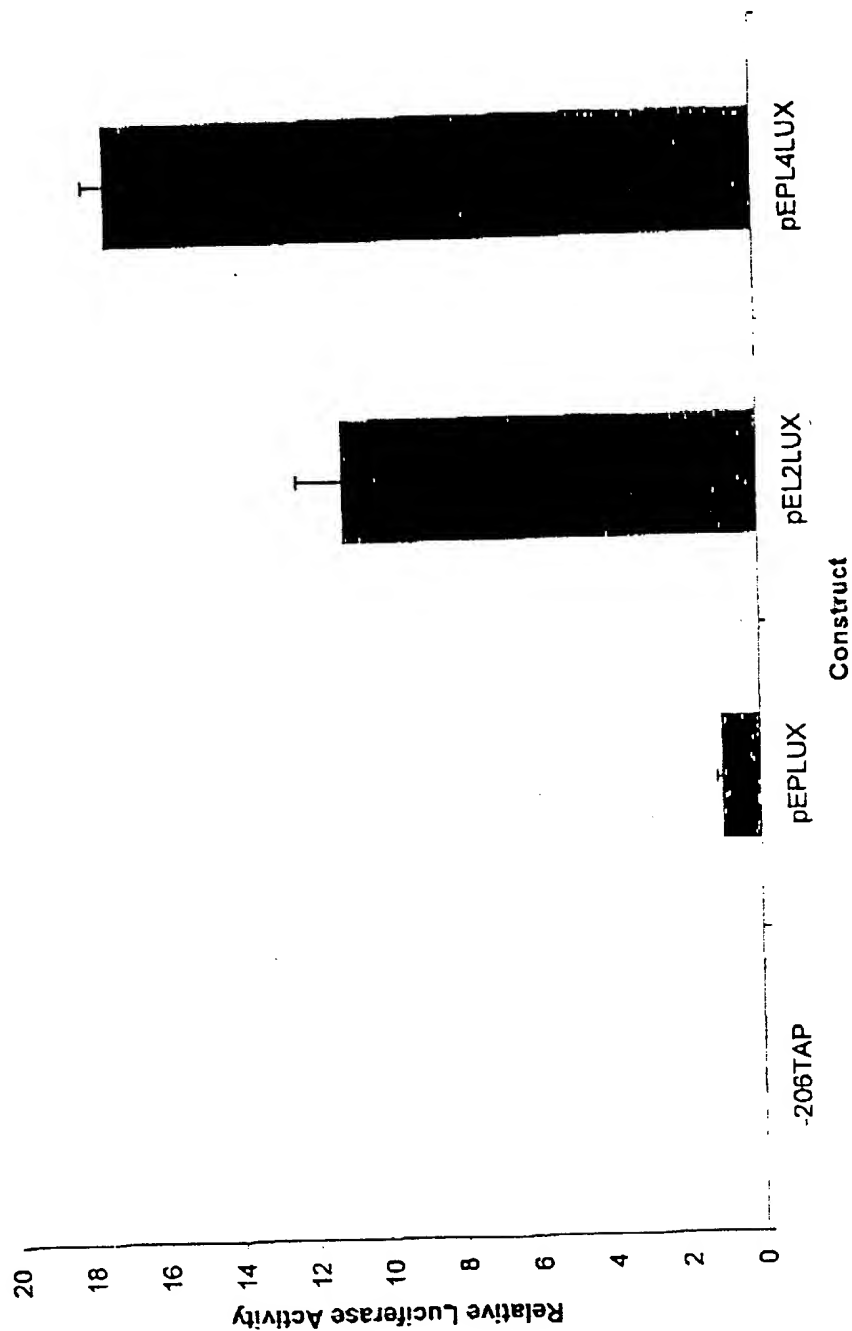


FIGURE 10B



FIGURE 11A

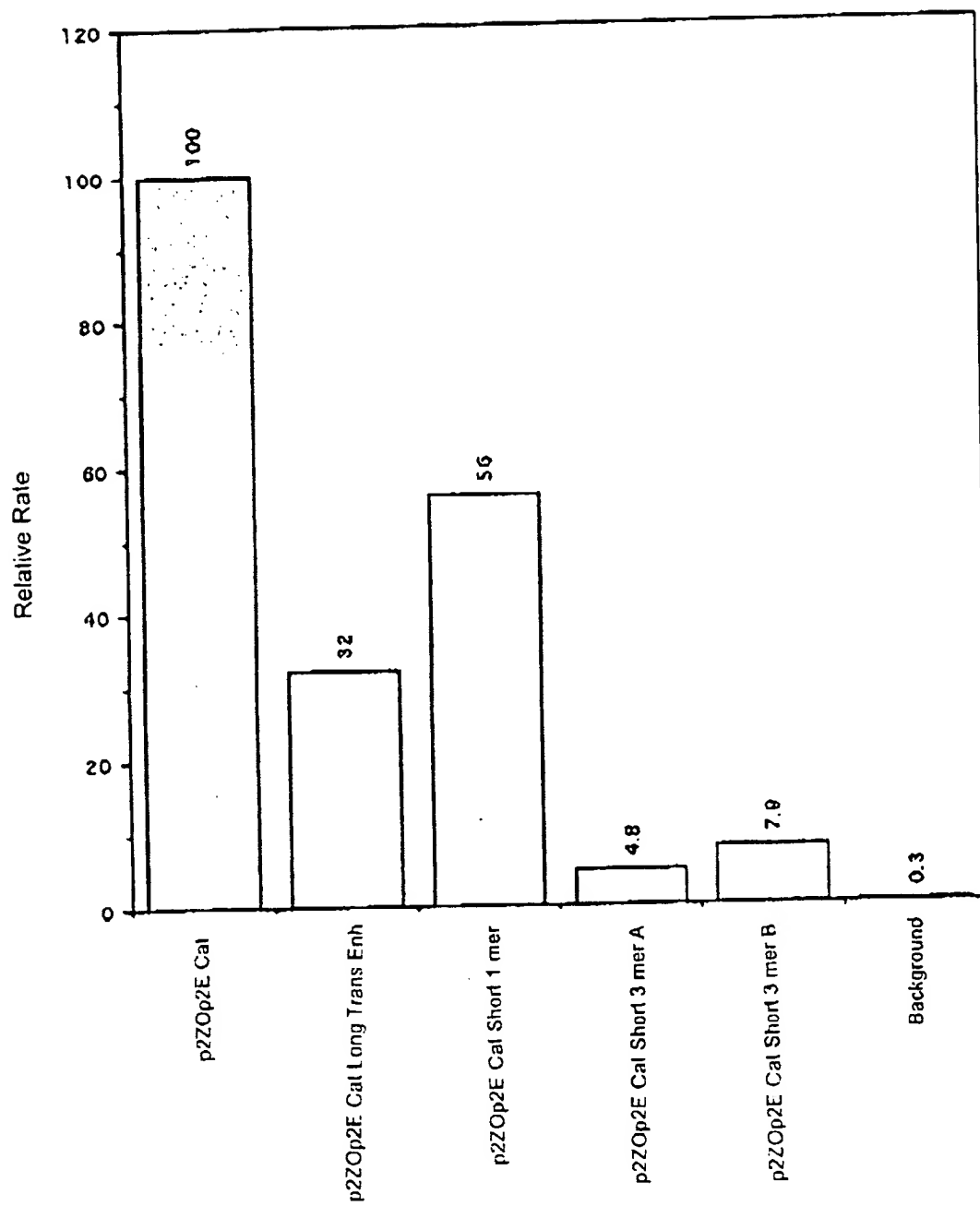


FIGURE 11B